

In re the application of:  
Eric Jervis and John Ramunas  
For: CULTURED CELL AND METHOD AND APPARATUS FOR CELL CULTURE

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**Amendments to the Claims**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (previously presented) A method of cell culture comprising:
  - confining a cell between first and second barriers, said barriers spaced at a distance comparable to the size of said cell to contact said cell and prevent said cell from traveling toward or away from each of said first and second barriers;
  - placing one or more spacers between said first and second barriers to prevent said first and second barriers over-compressing said cell; and
  - providing to said cell a culture substance.
2. (previously presented) The method of claim 1 wherein said first barrier is urged toward abutment with said second barrier and wherein said confining and said placing comprise at least partially separating said first and second barriers and introducing said cell and said one or more spacers between said first and second barriers.
3. (currently amended) The method of claim 1 ~~or claim 2~~ wherein said one or more spacers are rigid spheres, each having a diameter comparable to the size of said cell.
4. (currently amended) The method of ~~any one of claims~~ claim 1 ~~[[to 3]]~~ wherein each of said barriers has one or more characteristics selected to mimic the characteristics of the biological niche of said cell.
5. (currently amended) The method of ~~any one of claims~~ claim 1 ~~[[to 4,]]~~ wherein said providing comprises establishing a pre-selected concentration gradient of said culture substance within said space.

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6. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 5,] further comprising manually positioning a specific cell type adjacent said cell.

7. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 6,]] wherein said first and second barriers comprise first and second plates.

8. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 7,]] wherein a monolayer of cells are cultured between said barriers.

9. (original) The method of claim 8, further comprising removing a cell from said monolayer of cells when said cell to be removed is observed to meet one or more pre-determined criteria.

10. (original) The method of claim 9, wherein said one or more criteria are related to one or more of karyotype, morphology, and size.

11. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 10]] further comprising:  
limiting the number of cells surrounding said cell to permit control of properties of said cell.

12. (previously presented) The method of claim 11 wherein said cells surrounding said cell are selected to provide selected surfaces adjacent said cell.

13. (previously presented) The method of claim 2 wherein said introducing comprises introducing a suspension of said cell and said one or more spacers between said first and second barriers.

14. (currently amended) The method of claim 2 ~~or claim 13~~ further comprising fixing ends of said second barrier to said first barrier with an elastic fixative, said elastic fixative urging said first barrier toward abutment with said second barrier.

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15. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 14]] wherein said cell is confined between a surface of said first barrier and a surface of said second barrier, each surface selected to limit adhesion of said cell to said each surface.

16. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 15]] wherein said one or more spacers are configured to, with said first and second barriers, define a space having a shape mimicking the shape of the biological niche of said cell.

17. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 16,]] wherein said providing comprises flowing said culture substance to said cell at a predetermined rate.

18. (previously presented) The method of claim 7, wherein said plates are optically transparent.

19. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 18,]] wherein said providing comprises allowing said culture substance to permeate through one or both of said first and second barriers to between said first and second barriers.

20. (currently amended) The method of ~~any one of claims~~ claim 1 [[to 19]] further comprising sensing, with a sensor disposed adjacent a space between said first and second barriers, one or more of molecular concentration, temperature, osmolarity, pH, and shear force.

21. (previously presented) The method of claim 15 wherein said surface of said first barrier comprises a first type of molecules and said surface of said second barrier comprises a second type of molecules.

22. (currently amended) A method of forming an artificial tissue, comprising laying a first monolayer of cells on a second monolayer of cells and permitting cells of said first monolayer to interact with cells of said second monolayer wherein each one of said first and second monolayers is cultured ~~according to the method of any one of claims 1 to 21.~~ as follows:

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confining a cell between first and second barriers, said barriers spaced at a distance comparable to the size of said cell to contact said cell and prevent said cell from traveling toward or away from each of said first and second barriers;

placing one or more spacers between said first and second barriers to prevent said first and second barriers over-compressing said cell; and

providing to said cell a culture substance.

23. (previously presented) A combination of cell culture device and cell culture comprising:

first and second barriers and one or more spacers there-between defining a desired cell culture space said barriers contacting cells or bodies of cells constrained in said culture space wherein the spacers are sufficiently rigid to resist movement of said first and second barriers and approximate the size of said cells or bodies of cells such that the distance between said first and second barriers is comparable to the size of the cells or bodies of cells to be cultured therein and the spacers prevent said first and second barriers from overly compressing said cells or bodies of cells;

and means for providing to said culture space a culture substance at a predetermined rate.

24. (original) The combination of claim 23 wherein said means for providing to said space a culture substance comprises one or more fluid passageways allowing fluid communication to and from said space.

25. (original) The combination of claim 24, wherein said one or more fluid passageways comprise one or more microfluidic channels each terminating adjacent said space.

26. (currently amended) The combination of ~~any one of claims~~ claim 23 ~~[[to 25]]~~ further comprising means for regulating fluid flow to or from said space.

27. (currently amended) The combination of ~~any one of claims~~ claim 23 ~~[[to 26,]]~~ wherein at least one of said at least two barriers is permeable to nutrients and gases.

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28. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 27]] further comprising means for monitoring said cell constrained in said space.

29. (original) The combination of claim 28 wherein said means for monitoring comprises a sensor disposed in said chamber.

30. (original) The combination of claim 29 wherein said sensor is a sensor for sensing one or more of molecular concentration, temperature, osmolarity, pH, and shear force.

31. (currently amended) The combination of claim 29 ~~or claim 31,~~ further comprising one or more transparent electrodes for connecting said sensor to a control system.

32. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 31,]] wherein at least a portion of one of said barriers is transparent.

33. (original) The combination of claim 32, wherein said barriers comprise a microscope cover slip.

34. (original) The combination of claim 32, wherein said portion of said barrier is made of one of polystyrene, porous glass, or other contact lens materials.

35. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 34,]] wherein at least one of said barriers is moveable for adjusting the size of said space.

36. (original) The combination of claim 35, further comprising an actuator for moving said at least one of said barriers.

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37. (original) The combination of claim 36, wherein said actuator comprises one or more of an inflatable bladder, a screw, a lever, a clamp, a micrometer, and a piezoelectric crystal.

38. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 37]] wherein said one or more spacers are removable from said first or second barrier.

39. (currently amended) The combination of ~~any of claims~~ claim 23 [[to 37]] wherein said one or more spacers are molded on said first or second barrier.

40. (currently amended) The combination of ~~claims~~ claim 23 [[to 37,]] further comprising a divider for dividing said chamber into a plurality of regions and for preventing said cell from moving between said regions.

41. (original) The combination of claim 40, wherein said divider is removable from said container.

42. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 41,]] wherein the surfaces of said barriers comprise different types of molecules.

43. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 42,]] comprising a permeable membrane positioned to cover an opening adjacent said space for preventing said cell from leaving said space through said opening.

44. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 43,]] wherein said barriers defining a plurality of spaces for confining a plurality of cells therebetween.

45. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 44,]] which is included in a cartridge.

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46. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 45,]] further comprising said fluid culture medium which is received in said chamber and immerses said cell.

47. (original) The combination of claim 46, wherein at least one wall of said container has a septum allowing access to said space with a syringe or a pipette.

48. (currently amended) The combination of ~~any one of claims~~ claim 23 [[to 47,]] further comprising a capillary conduit for transporting a cell-containing fluid to or from said space.

49. (original) A combination of a cell culturing device and a cell culture, comprising:

a container defining a chamber for receiving a fluid culture medium;

at least two barriers defining a space in said chamber, each one of said barriers having one or more pre-selected characteristics;

an assembly of two or more cells constrained in said space so as to keep said assembly therein and in continuous contact with each said barriers during culturing; and

means for providing to said space a culture substance at a predetermined rate

50. (original) The combination of claim 49 wherein said space is sufficiently small to permit control of properties of individual cells in culture.

51. (currently amended) The combination of claim 49 ~~or claim 50~~ wherein said assembly comprises a monolayer of cells.

52. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 51]] wherein said means for providing to said space a culture substance comprises one or more fluid passageways allowing fluid communication to and from said space.

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53. (original) The combination of claim 52, wherein said one or more fluid passageways comprise one or more microfluidic channels terminating adjacent said space.

54. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 53]] further comprising means for regulating fluid flow to or from said space.

55. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 54,]] wherein at least one of said at least two barriers is permeable to nutrients and gases.

56. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 55]] further comprising means for monitoring said cell constrained in said space.

57. (original) The combination of claim 56 wherein said means for monitoring comprises a sensor disposed in said chamber.

58. (original) The combination of claim 57 wherein said sensor is a sensor for sensing one or more of molecular concentration, temperature, osmolarity, pH, and shear force.

59. (currently amended) The combination of claim 57 ~~or claim 58~~, further comprising one or more transparent electrodes for connecting said sensor to a control system.

60. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 59,]] wherein at least a portion of one of said barriers is transparent.

61. (original) The combination of claim 60, wherein one of said barriers is a microscope cover slip.

62. (original) The combination of claim 61, wherein said portion of said barrier is made of one of polystyrene, porous glass, or other contact lens materials.



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63. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 62,]] wherein at least one of said barriers is moveable for adjusting the size of said space.

64. (original) The combination of claim 63, further comprising an actuator for moving said at least one of said barriers.

65. (original) The combination of claim 64, wherein said actuator comprises one or more of an inflatable bladder, a screw, a lever, a clamp, a micrometer, and a piezoelectric crystal.

66. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 65,]] further comprising one or more spacers placed between said barriers for preventing said barriers over-compressing said cell.

67. (original) The combination of claim 66, wherein said one or more spacers are molded on one or more of said barriers.

68. (currently amended) The combination of ~~claims~~ claim 49 [[to 67,]] further comprising a divider for dividing said chamber into a plurality of regions and for preventing said cell from moving between said regions.

69. (original) The combination of claim 68, wherein said divider is removable from said container.

70. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 69,]] wherein the surfaces of said barriers comprise different types of molecules.

71. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 70,]] comprising a permeable membrane positioned to cover an opening adjacent said space for preventing a cell from leaving said space through said opening.

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72. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 71,]] wherein said barriers defining a plurality of spaces for confining a plurality of assembly of cells therebetween.

73. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 72,]] which is included in a cartridge.

74. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 73,]] further comprising said fluid culture medium which is contained in said chamber and immerses said assembly of cells.

75. (original) The combination of claim 74, wherein at least one wall of said container has a septum allowing access to said space with a syringe or a pipette.

76. (currently amended) The combination of ~~any one of claims~~ claim 49 [[to 75,]] further comprising a capillary conduit for transporting a fluid to or from said space.

77. (currently amended) An assembly of cells comprising a cell cultured in accordance with the following method; ~~of any one of claims 1 to 21~~.

confining a cell between first and second barriers, said barriers spaced at a distance comparable to the size of said cell to contact said cell and prevent said cell from traveling toward or away from each of said first and second barriers;

placing one or more spacers between said first and second barriers to prevent said first and second barriers over-compressing said cell; and

providing to said cell a culture substance.

78. (currently amended) A cell cultured in accordance with the following method; ~~of any one of claims 1 to 21~~.

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confining a cell between first and second barriers, said barriers spaced at a distance comparable to the size of said cell to contact said cell and prevent said cell from traveling toward or away from each of said first and second barriers;

\_\_\_\_\_ placing one or more spacers between said first and second barriers to prevent said first and second barriers over-compressing said cell; and

providing to said cell a culture substance.

79. (currently amended) The assembly of cells of claim 77 ~~or the cell of claim 78~~ for use as an artificial tissue, organ, cell transplant, or *in vitro* fertilization.

80. (previously presented) A apparatus for culturing cells in a controlled environment comprising:

i) first and second barriers and one or more spacers there-between defining a desired confined space wherein the distance between the barriers is comparable to the size of cells or bodies of cells to be cultured therein, the barriers contacting said cells or bodies of cells impeding their movement wherein said spacers are sufficiently rigid to resist movement of said first and second barriers;

and prevent first and second barriers from overly compressing said cells or bodies of cells.

ii) the inner surface of one or both of said first and second barriers having one or more characteristics or properties selected to mimic the characteristics of the biological environment of said cells;

iii) means for providing a culture substance to said space.

81. (original) The apparatus of claim 80 wherein said barriers comprise two opposing glass plates.

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82. (currently amended) The apparatus of claim 80 ~~or claim 81~~, wherein said space has a size comparable to the size of a single cell.

83. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 82,]] wherein said space limits cells cultured therein to a monolayer.

84. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 83]] wherein said means for providing comprises one or more fluid passageways allowing fluid communication to and from said space.

85. (original) The apparatus of claim 84 wherein said one or more fluid passageways comprise one or more microfluidic channels terminating adjacent said space.

86. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 85]] further comprising means for regulating fluid flow to or from said space.

87. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 86]] wherein at least one of said barriers is permeable to nutrients and gases.

88. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 87]] further comprising means for monitoring a cell constrained in said space.

89. (original) The apparatus of claim 88 wherein said means for monitoring comprises a sensor disposed in said chamber.

90. (original) The apparatus of claim 89 wherein said sensor is a sensor for sensing one or more of molecular concentration, temperature, osmolarity, pH, and shear force.

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91. (currently amended) The apparatus of claim 89 ~~or claim 90~~, further comprising one or more transparent electrodes for connecting said sensor to a control system.

92. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 91,]] wherein at least a portion of one of said barriers is transparent.

93. (original) The apparatus of claim 92, wherein one of said barriers is a microscope cover slip.

94. (original) The apparatus of claim 93, wherein said portion of said barrier is made of one of polystyrene, porous glass, or other contact lens materials.

95. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 94,]] wherein at least one of said barriers is moveable for adjusting the size of said space.

96. (original) The apparatus of claim 95, further comprising an actuator for moving said at least one of said barriers.

97. (original) The apparatus of claim 96, wherein said actuator comprises one or more of an inflatable bladder, a screw, a lever, a clamp, a micrometer, and a piezoelectric crystal.

98. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 97]] wherein said one or more spacers are removable from said first or second barrier.

99. (currently amended) The apparatus of ~~any one of claims~~ claim 80 [[to 97]] wherein said one or more spacers are molded on said first or second barrier.

100. (currently amended) The apparatus of ~~claims~~ claim 80 [[to 98,]] further comprising a divider for dividing said chamber into a plurality of regions and for preventing said cell from moving between said regions.

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101. (previously presented) The apparatus of claim 99, wherein said divider is removable from said container.

102. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 101~~, wherein the surfaces of said barriers comprise different types of molecules.

103. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 102~~, comprising a permeable membrane positioned to cover an opening adjacent said space for preventing a cell from leaving said space through said opening.

104. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 103~~, wherein said barriers defining a plurality of spaces for confining a plurality of cells therebetween.

105. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 104~~, which is included in a cartridge.

106. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 105~~, further comprising said fluid culture medium which is contained in said chamber.

107. (original) The apparatus of claim 106, wherein at least one wall of said container has a septum allowing access to said space with a syringe or a pipette.

108. (currently amended) The apparatus of ~~any one of claims~~ claim 80 ~~to 107~~, further comprising a capillary conduit for transporting a fluid to or from said space.

109. (cancelled)

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110. (cancelled)

111. (original) A method of cell culturing, comprising:

culturing one or more cells while restricting movement of said one or more cells such that each one of said one or more cells is in continuous contact with two opposing barrier surfaces and is mobile between said barrier surfaces.

112. (original) The method of claim 111, wherein said barrier surfaces are generally parallel with each other.

113. (currently amended) The method of claim 111 ~~or claim 112~~, wherein said barrier surfaces are generally planar.

114. (currently amended) The method of ~~any one of claims~~ claim 111 ~~to 113~~, further comprising, during said culturing, obtaining one or more images of said one or more cells.

115. (original) The method of claim 114, wherein said one or more images are obtained using a non-confocal imaging device.

116. (original) The method of claim 114, wherein said one or more images are obtained using a bright field imaging device or a fluorescent imaging device.

117. (original) The method of claim 116, wherein said one or more images are obtained using a differential interference contrast (DIC) imaging device.

118. (currently amended) The method of ~~any one of claims~~ claim 114 ~~to 117~~, wherein said one or more images comprise a plurality of images taken over a period of time longer than about one day.

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119. (currently amended) The method of ~~any one of claims~~ claim 114 ~~to 117~~, wherein said one or more images comprise a plurality of images taken over a period of time which is not limited by significant or substantial phototoxic effects to impose a constraint for gathering information on behavior histories of a cell or cells.